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Stability-Indicating Assay Method for Determination of Rosuvastatin in Nano-Formulation and Pharmaceutical Dosage form By RP-HPLC

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Abstract : A simple, precise, isocratic, reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the rapid determination of rosuvastatin using Kromasil C-18, 4.6 x 250 mm (id), 5 µm HPLC column. The run-time was 8 min with retention time of rosuvastatin at 4.72 min. RP-HPLC method was validated according to ICH guidelines with respect to linearity, accuracy, precision and robustness. The limit of detection and limit of quantitation was found to be 0.17 and 0.7µg/ mL, respectively. Developed method was found to be linear in the concentration range of 1.56 to 50 µg/ mL with regression coefficient of 0.9998. Further, the proposed method was found to be reproducible and convenient for stability indicating analysis of rosuvastatin in marketed tablet dosage form and developed solid lipid nanoparticles.

Keywords : Rosuvastatin; Forced degradation studies; RP-HPLC; Stability-indicating.

Introduction

Rosuvastatin Calcium is a synthetic drug approved by US-FDA in 2003 for the treatment of dyslipidemia. Its therapeutic activity is mediated by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and prevents conversion of HMG-CoA to mevalonate[1]. It is sparingly soluble in water and methanol, and slightly soluble in ethanol. Various analytical methods have already been reported in literature for the analysis of rosuvastatin such as Spectrophotometric methods[2-6], High performance thin layer chromatography (HPTLC)[7], High performance liquid chromatography (HPLC)[8-13] and Ultra performance liquid chromatography (UPLC)[14]. The present study aimed to develop a simple, sensitive, precise and accurate stability-indicating RP-HPLC method for the determination of ROS in tablet dosage form and lipid based nano-formulation.

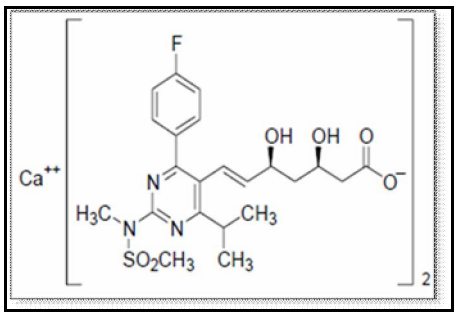

Fig. 1. Chemical structure of Rosuvastatin Calcium

Experimental

Reagents and chemicals

Rosuvastatin Calcium was received as a gift sample from Astra Zeneca (India) Pvt. Ltd. All other chemicals and solvents used throughout the study were HPLC/ AR grade. Acetonitrile, hydrochloric acid (HCl), Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from s.d.Fine Chemicals Ltd., Mumbai, India. A MilliQ plus water purification system (Miliford, USA), was used to prepare de-ionized water (>18 μΩ).

Instrumentation and analytical conditions[15]

Analysis of rosuvastatin was carried out on HPLC instrument (Agilent Technologies, 1220 Infinity LC) equipped with binary pump, an auto sampler, photodiode array (PDA) detector and LCchrom software. Chromatographic analysis was performed in isocratic elution mode using Kromasil C-18, 4.6 x 250 mm (id), 5 μm HPLC column and mobile phase consisting of buffer pH 4.8 (0.78%w/v of sodium dihydrogen orthophosphate in deionized water) and acetonitrile in a ratio of 50:50v/v.Prior to use, the mobile phase was filtered through 0.45 μ membrane. The flow rate was 1.0 mL/ min with injection volume of 20 μL and the detection wavelength was set at 241 nm.

Preparation standard solution of ROS

A quantity of rosuvastatin calcium equivalent to 25 mg rosuvastatin was measured and transferred to 25 mL volumetric flask. Volume was made up with mobile phase to obtain a primary stock solution of concentration 1000 μg/mL. The primary stock solution was suitably diluted with the mobile phase to obtain secondary stock solution of 100 μg/ mL of rosuvastatin. An aliquot of this stock was diluted with mobile phase to get standard working solution of the concentration 12.5 μg/mL.

Optimization of RP-HPLC method[15]

Selection of mobile phase

Mobile phase was selected on the basis of good resolution, peak purity, peak symmetry, theoretical plates etc. Different solvents in various ratios were used to obtain a peak of rosuvastatin which met all system suitability parameters.

System suitability study

System suitability for analysing rosuvastatin at a concentration of 12.5 μg/mL was assessed by spiking the sample in triplicate. Parameters such as peak symmetry (symmetry factor), theoretical plates of the column (N) and retention time (R_t) were evaluated.

Forced degradation studies[16-18]

Forced degradation studies provides information about the possible degradation of drug during stability studies and specificity of the method. Prior to analysis, 1000 μg/mL of rosuvastatin solution in mobile phase was prepared. To 1mL of above stock solution of rosuvastatin, 1mL of 1 N HCl and 5 N NaOH were added separately. These mixtures were refluxed separately at 80ºC for 24 h.The resultant solutions were diluted with mobile phase to give solutions containing rosuvastatin in the concentration of 100μg/ mL The solutions were neutralized before spiking into the HPLC system. Similarly, oxidative degradation (3%v/v H_2O_2, reflux at 80ºC for 24 h), thermal degradation in wet state (80°C for 24 h) and photo degradation in dry and wet state (UV light of wavelength 254 nm for 20 h) were also conducted. Solutions of rosuvastatin under each stress condition were analysed bydevelopedRP-HPLC method. Peak area of samples under each condition was compared with peak areaof standard rosuvastatin sample and the percent degradation was calculated using equation (1).

$$Percent\ degradation = \frac{Peak\ area\ of\ ROS\ under\ stress\ condition}{Peak\ area\ of\ standard\ ROS} \times 100 \quad (1)$$

Method validation[19-24]

Linearity and range

Linearity for the developed method was obtained by preparing solutions containing various concentrations ofrosuvastatin. From the secondary stock solution (as mentioned above), required amounts of aliquots were withdrawn and diluted with mobile phase to obtain concentrations in the range 1.56-50 µg/mL (n=3). The resulting solutions were subjected to RP-HPLC analysis; peak areas were determined and linearity plot was constructed. Linearity range was determined from the regression coefficient value (r^2).

Limit of detection (LoD) and limit of quantitation (LoQ)[25]

LoD and LoQ were calculated using equations, 3.3 σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of Y-intercept.

Precision

a) Repeatability

Sequential, repetitive six injections (n=6) of ROS solution (12.5 µg/ mL) in mobile phase was subjected to RP-HPLC analysis and their peak responses were examined. Percent relative standard deviation (%RSD) of peak areas of six injections was computed.

b) Intermediate precision

Intermediate precision study was carried out as intraday study. For the study, sample solutions of rosuvastatin from six different weighing's were subjected to RP-HPLC analysis at three time points in a day and their peak responses were examined. The %RSD for 6 assay values for three time points was calculated.

Accuracy

Accuracy of the developed method was determined by recovery study. Three levels i.e. 80, 100 and 120% of test concentration were chosen for experimental purpose. Placebo blend of nano-formulation was prepared and then spiked with known amount of rosuvastatin. Each concentration level was performed in triplicate. Percent recovery and percent RSD were calculated.

Robustness

Robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal use. To study the effect of pH on the peak area, pH was changed by ±0.2 units while the other analytical parameters were held constant. The effect of change in flow-rate on resolution was studied by varying the flow-rate by ±0.1 units.

Applications of the developed method[26-28]

Analysis of rosuvastatin from marketed tablets

To determine the content of rosuvastatin in marketed tablets (label claim: 5 mg of rosuvastatin per tablet), the tablets were crushed and transferred into 50 mL of volumetric flask containing mobile phase. To ensure complete extraction of drug, the sample was sonicated for 15 min. Volume was made up to the final volume and the resulting solution was filtered through 0.45 µ membrane. The solutions were suitably diluted and spiked in the chromatogram. Similar procedure was followed for determination of uniformity of content of 20 marketed tablets (Rosuvas 5).

Analysis of rosuvastatin from solid lipid nanoparticles

To determine the content of rosuvastatin in lyophilized solid lipid nanoparticles (label claim: 5 mg of rosuvastatin per 350 mg of lyophilized solid lipid nanoparticles), nanoparticles equivalent to 5 mg of rosuvastatin was accurately weighed and transferred into 50 mL of volumetric flask containing mobile phase

(n=6). To ensure complete extraction of drug, the sample was sonicated for 15 min. Volume was made up to the final volume and the resulting solution was filtered through 0.45 µ membrane. The solutions were suitably diluted with mobile phase to obtain concentration in the linearity range. The assay procedure was repeated 6 times and average percentage of drug in the formulation was calculated. The possibility of excipient interference in the analysis was studied.

Results and Discussion

Selection of mobile phase

Mobile phase consisting of buffer pH 4.8 (0.78%w/v of sodium dihydrogen orthophosphate in deionized water) and acetonitrile in a ratio of 50:50 v/v at 1 mL/min flow rate was found to be suitable in obtaining a resolved peak of rosuvastatin at 4.72 min. A typical chromatogram of rosuvastatin in optimized chromatographic conditions is shown in **Fig. 2**. Higher concentration of aqueous phase in the buffer increased the retention time to more than 10 min; while higher concentration of organic phase in the buffer resulted in elution of the peak at the solvent front. Nonetheless, equivalent proportion of organic to aqueous phase lead to rapid determination of the drug peak without interference from solvents.

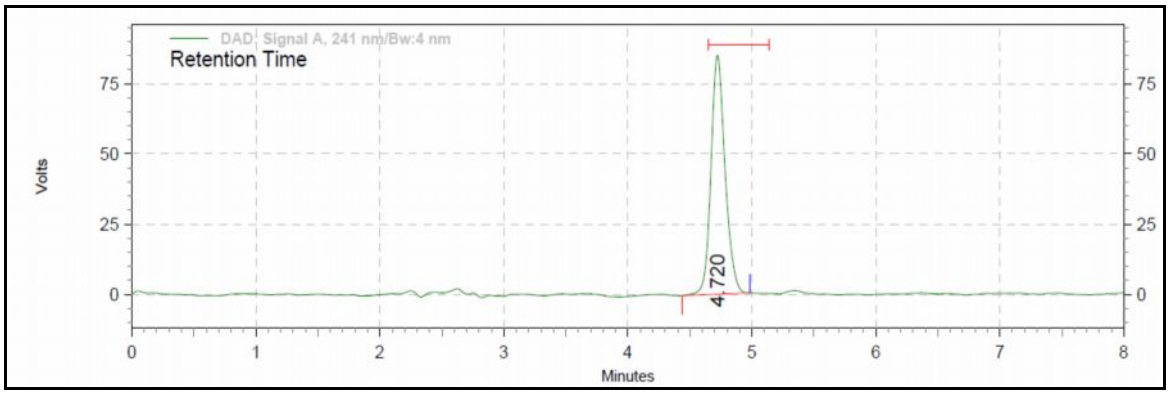

Fig. 2. Representative chromatogram of ROS (12.5 µg/mL) (R_t = 4.72 min)

System suitability study

System suitability parameters were found to be in compliance with USP guidelines showing theoretical plates (N) of 8528 (with acceptable limit:more than 2000) and tailing factor of 1.178 (with acceptable limit:less than 2).

Forced Degradation Studies

Initially, a typical chromatogram of rosuvastatin of 100 µg/mL concentration was obtained to relate the percent degradation of the stress-induced drug (**Fig. 3)**. During acid hydrolysis, rosuvastatin refluxed for 24 h with 1 NHCl showed appearance of degradation peaks at 5.85, 7.37, 10.27 and 10.84 min **(fig. 4)**. About 42.55 % degradation of rosuvastatin was observed in acidic condition. During alkaline hydrolysis, rosuvastatin refluxed for 24 h with 5 N NaOH showed degradation of 8.5%indicating stability of the drug in basic conditions **(fig. 5)**. Rosuvastatin was observed to be liable to oxidation (about 75 % degradation) at 3% H_2O_2 at room temperature for 24 h. The degradants were eluted at 4.07, 5.87 and 6.61 min**(fig. 6)**. During thermal degradation (treatment to wet heat at 80°C for 24 h) the chromatogram of rosuvastatin did not show appearance of any degradation peak**(fig. 7)**. This reflected the stability of drug to heating conditions encountered due to formulation methodologies such as solvent emulsification-evaporation technique or high pressure homogenization employed for preparation of solid lipid nanoparticles. During photo degradation (exposed to UV light at both wet and dry condition for 20 h), degradation peaks at 5.6, 5.98 and 6.53 min **(fig. 8 & 9)** were observed with higher intensity in wet condition. The amount of degradation of rosuvastatin in wet and dry condition was found to be 85% and 14.6%, respectively. Thus from the forced degradation study, it can be concluded that rosuvastatin shows vulnerability to acid hydrolysis, oxidation and photodegradation. However, it was observed to be stable inalkaline and thermal condition. The degradation peaks were found to be well

separated from the drug peak. The method developed is able to determine rosuvastatin in presence of its degradation products and hence can be said to be specific for determination of rosuvastatin.

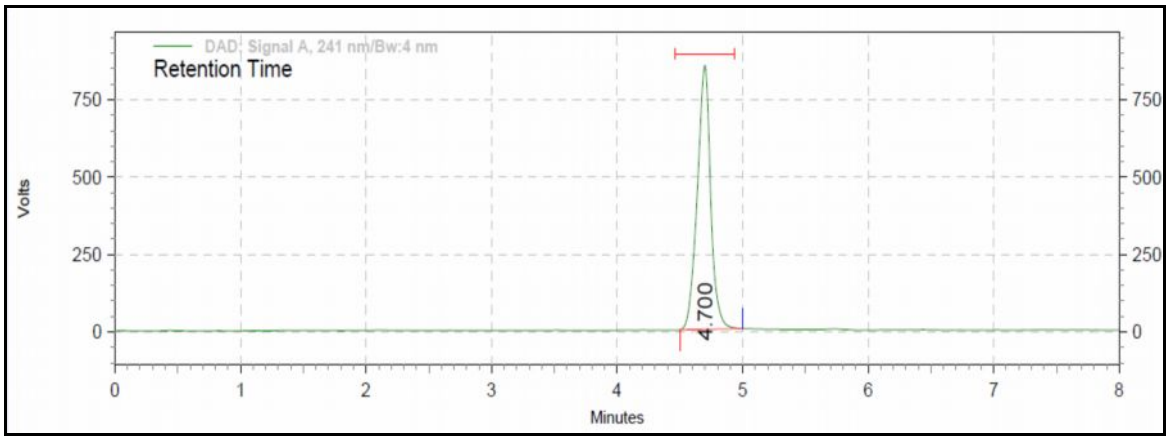

Fig. 3. Representative chromatogram of rosuvastatin (100 µg/mL)

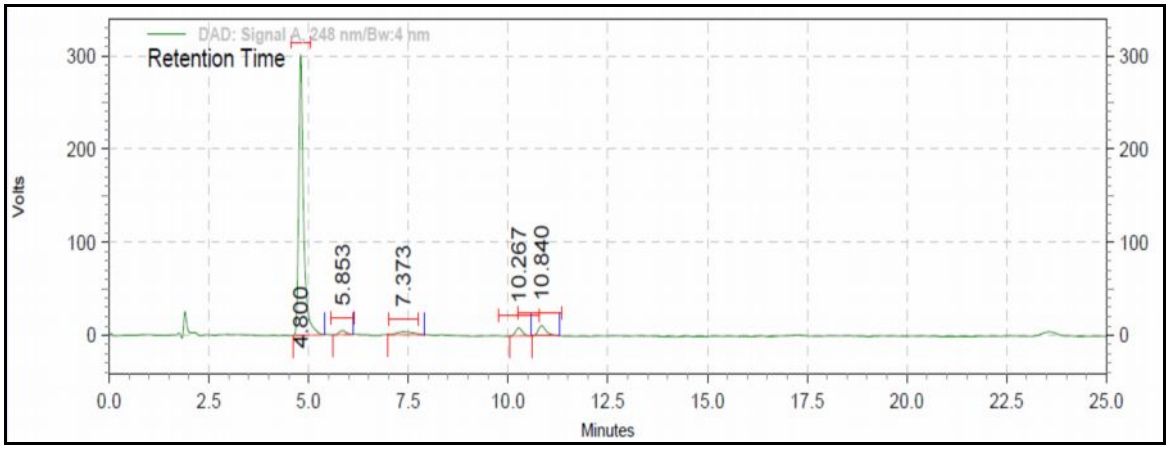

Fig. 4. Acidic degradation of rosuvastatin (1 N HCl, 24 h reflux at 80ºC)

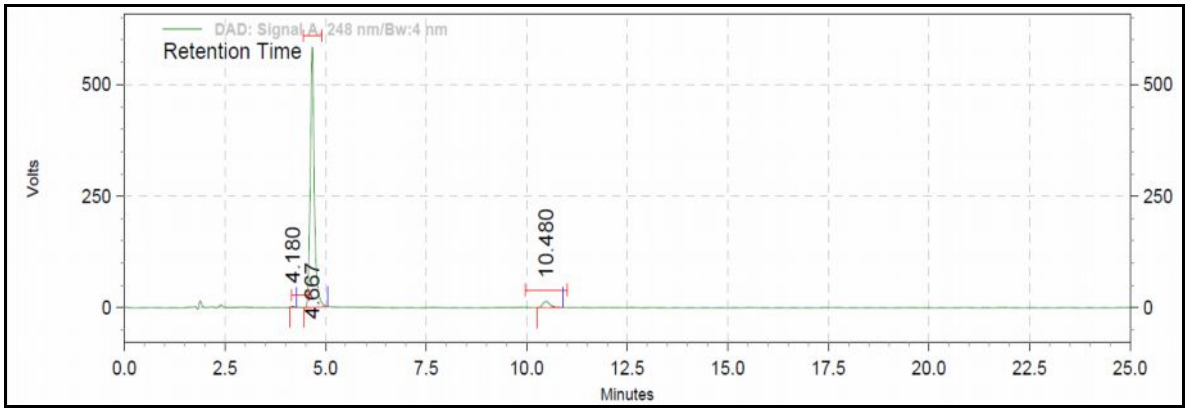

Fig. 5. Alkaline degradation of rosuvastatin (5 N NaOH, 24 h reflux at 80ºC)

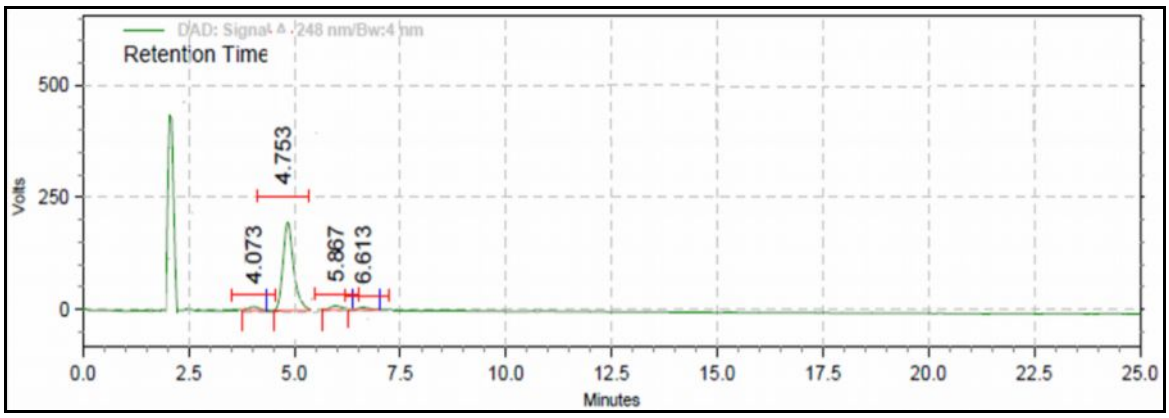

Fig. 6. Oxidative degradation of rosuvastatin (3% H_2O_2 for 24 h reflux at 80ºC)

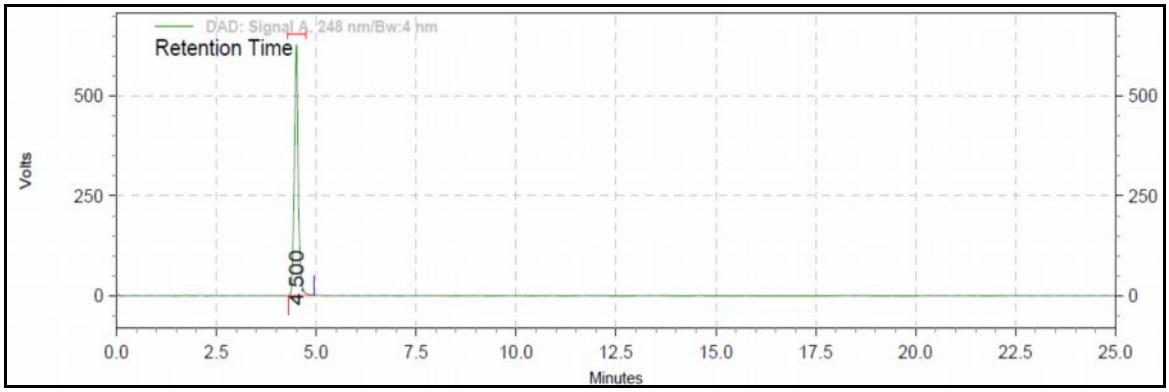

Fig. 7. Thermal degradation of rosuvastatin in wet condition (exposed to 80 °C for 24 h)

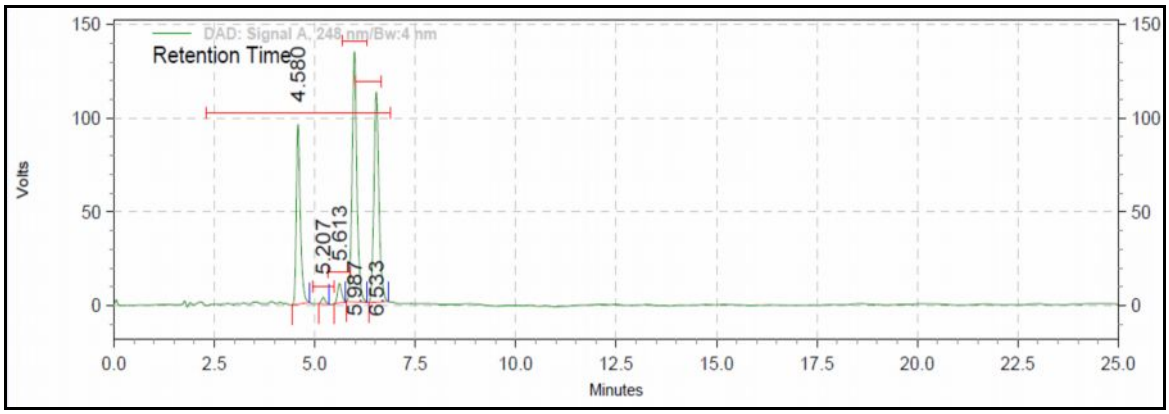

Fig. 8. Photodegradation of rosuvastatin in wet condition (exposed to UV light of wavelength 254 nm for 20 h)

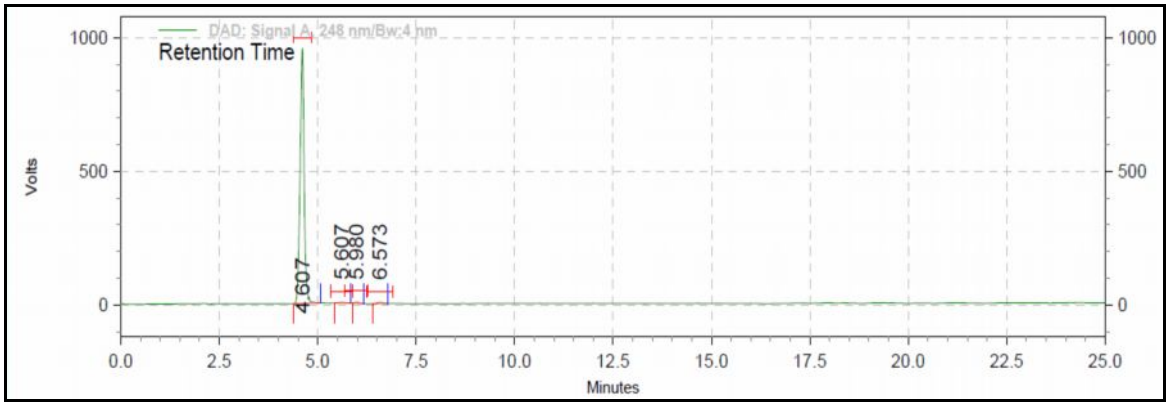

Fig. 9. Photodegradation of rosuvastatin in dry condition (exposed to UV light of wavelength 254 nm for 20 h)

Validation

Linearity and range[29]

Linearity of rosuvastatin**(fig. 10)** in mobile phase was plotted in the range of 1.56-50 µg/mL and range was studied in 80-120 % of its test concentration (12.5 µg/mL). Typical chromatograms of rosuvastatin in the linearity range are shown in **fig. 11**. Developed method was found to be linear in the selected concentration range with regression coefficient of 0.9998, slope and intercept values being 108496 and 21659, respectively.

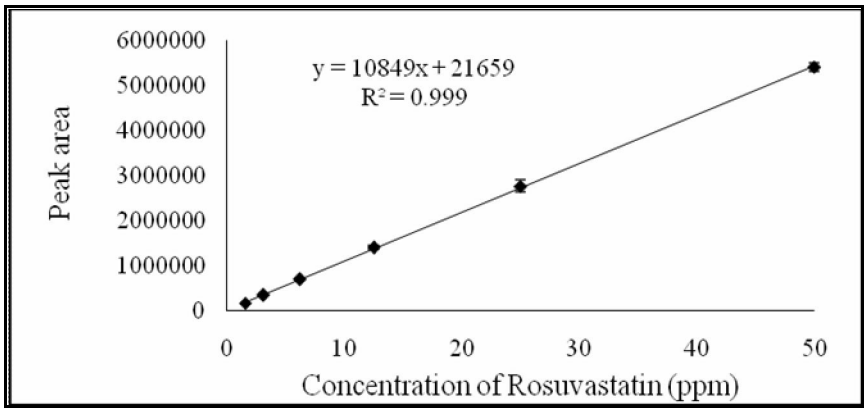

Fig. 10. Linearity plot of Rosuvastatin

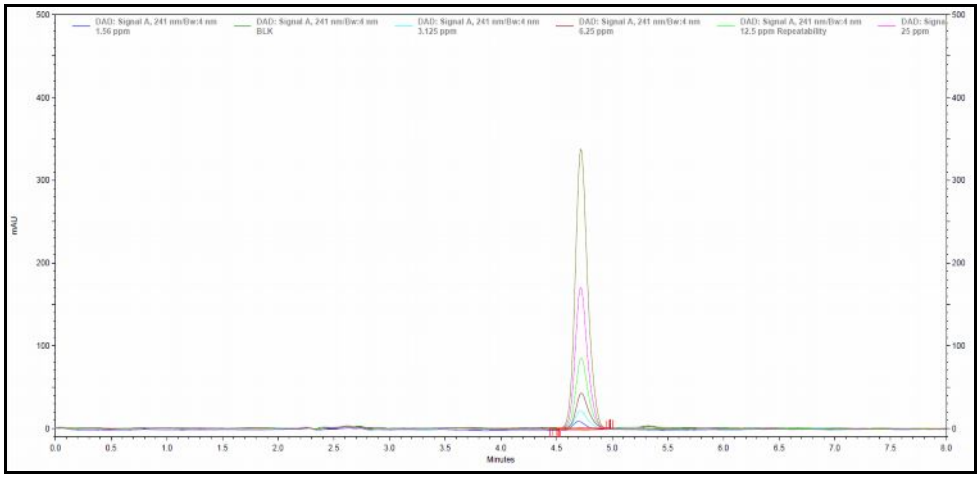

Fig. 11. Chromatograms of Rosuvastatin in the range of 1.56-50 µg/mL

LoD and LoQ

LoD and LoQ were found to be 0.17 and 0.7 µg/mL, respectively, computed by using ANOVA analysis.

Precision

Percent relative standard deviation (RSD) of area of sequential, repetitive six injections of standard rosuvastatin solution (12.5 µg/ mL) was found to be 0.76%, which meets the system suitability criterion of percent RSD NMT 2%.

Intermediate precision which expresses within laboratory variations was evaluated at three time points in a day and their peak responses were examined. Percent RSD of drug content of all 18 samples was found to be 0.63%, which is below 2% confirming that the method was precise.

Accuracy

An excellent recovery of 98-102% with RSD value of 0.92 was obtained at all the experimental levels (80, 100 and 120%). Hence, it was inferred that there was no interference in quantitative estimation of rosuvastatin and proposed RP-HPLC method is proved to be accurate.

Robustness

Deliberate change of mobile phase pH and flow-rate of the mobile phase through the column showed % RSD values of less than 2 reflecting robustness of the method (Table 1).

Table 1. Effect of change in pH and flow-rate on % assay of rosuvastatin

Condition	Retention time*	Mean Area*	Assay (%)*
Unaltered condition	4.72	1406063	100.98
Altered condition			
pH of buffer (4.6)	4.71	1381339	99.20
pH of buffer (5.0)	4.75	1391187	99.91
Flow rate 0.9 mL/ min	4.97	1399962	100.54
Flow rate 1.1 mL/ min	4.71	1386962	99.61
* Values are mean of three determinations (n=3)			

From the above table it can be seen that there was no significant change in the % assay of rosuvastatinindicating that the method is robust.

Applications of the developed method

Analysis of rosuvastatin from marketed tablets

From the chromatogram of rosuvastatin samples extracted from marketed rosuvastatin tablets showed a distinct peak of rosuvastatin with no interference from tablet excipients. The drugcontent was found to be 100.80 with %RSD of 0.73. Low %RSD value indicated suitability of the developedmethod for routine analysis of rosuvastatin in pharmaceutical dosage forms.

Analysis of rosuvastatin from lyophilized solid lipid nanoparticles

In the chromatogram of the rosuvastatin samples extracted from lyophilized solid lipid nanoparticles, a defined peak of rosuvastatin was observed with no interference from excipients used to develop SLN such as lipids, surfactants etc. The drug content was found to be 99.86 with %RSD of 1.2. Low %RSD value indicated suitability of the developed method for routine analysis of rosuvastatin in nano-formulations.

Conclusion

The developed RP-HPLC method is precise, specific, accurate and stability indicating for the rapid determination of rosuvastatin. The stress testing results revealed that the method is was selective and stability indicating. The developed method has presented the ability to separate rosuvastatin from its degradants. Thus the method can be useful in analysing rosuvastatin samples obtained during accelerated stability studies. Results of forced degradation studies also provides information on the type of excipients suitable during the formulation stage.The developed method is versatile foranalysis of rosuvastatin in nano-formulations and marketed rosuvastatin tablets. The method was specific since vehicles in the formulation did not interfere in the estimation of rosuvastatin. The amount of drug that was analysed was found to be in good agreement with the label claim of formulations.

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